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PURDUE UNIVERSITY.

Agricultural Experiment Station.

BULLETIN NO. 59. VOL. VII.
MARCH, 1896.

Bacteriosis of Carnations.

Published by the Station:
LAFAYETTE, IND.
U. S. A.

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BULLETINS OF 1896 ISSUED TO DATE.

No. 58. Hog cholera and swine plague in Indiana. February.
No. 59. Bacteriosis of carnations.*

*Not for general distribution.

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BACTERIOSIS OF CARNATIONS.

By J. C. Arthur and H. L. Bolley.

The disease which is treated at some length in this bulletin is quite familiar to many florists at the present time, owing to preliminary notices* of the researches herein detailed having at different times been given orally before a number of societies, and in part been published. But when the investigation was begun in 1888, and up to the time the most important conclusions had been attained and made known, no such disease had been recognized by either the scientist or the cultivator. It was so obscure that the literature of carnations previous to 1890 shows no allusion that can reasonably be construed as referring to this disease.

Anomalous as it may seem, the disease which we are now calling bacteriosis, and find to be the most abundant and ever-present of the maladies of the carnation plant, easily detected even in its earlier stages, and so common as to be to some extent almost a constant accompaniment of the plant, was not previously known to the cultivator, although it was before his eyes continually and when at its worst caused the foliage of his plants to turn a sickly yellow, or to dry up and die, and the crop of flowers to become greatly curtailed on account of it. It is, however, much to the credit of the florist, and deeply gratifying to the investigator, that having had his attention called to the matter, he at once became interested, and in many instances mastering all that was to be learned, modified his methods and adopted remedies that have essentially banished the disease from the best greenhouses.

Not to anticipate further, however, this introduction may be closed by saying that the study now described in full for the first time, beside making known to the botanist an interesting plant disease caused by true parasitic bacteria, has already led to results of great practical and economical importance for the florist, and may be expected to serve further good ends when better and more fully known.

General description of the disease.—Bacteriosis is a disease of the carnation leaf, rarely attacking the stem or other parts of the plant.

*Arthur, J. C., Proc. Amer. Assoc. Adv Sci., xxxviii (1889) p. 280; (name erroneously printed "Harter") *Amer. Florist*, vi (1891) p. 419; Rep. Amer. Carn. Soc. 1892, p. 52; Rep Amer. Carn. Soc. 1894, p. 12; *American Florist*, ix (1894), p. 647.

See also Halsted, B. D., *Amer. Florist*, viii (1893), p. 732, 2 illustrations; Rep. Amer. Carn. Soc. 1893, p. 57.

It generally starts in the leaf when immature, and is best diagnosed in the younger but full-sized leaves nearest the upper end of the stem. Taking such a leaf, which on its surface presents no unusual appearance to the eye, and holding it toward a strong light, small pellucid dots may be detected scattered irregularly through the leaf, sometimes having a faint yellowish color which are the centers of infection (see fig. 1). The appearance of the dots has a close resemblance to those of the oil glands in the leaves of the common St. John's-wort (*Hypericum perforatum*), a rather abundant weed, or in the leaves of the false indigo (*Amorpha fruticosa*), a native shrub, except that they have no regular disposition. Sometimes the surface of the leaf is slightly raised over the dots, making watery pimples.

After a time the surface of the leaf above the dots changes enough to indicate their presence, and finally shows a distinct spot. As the disease extends inside the leaf, the surface tissues dry, the internal tissues collapse, and whitish sunken spots appear. In some colored varieties of carnation the spots vary somewhat by being more or less reddish or purplish.* As the spots increase in size the leaves wither, still clinging to the stem. Such spots never show distinct central darker colored specks, and rarely any concentric circles, as do the spots made by parasitic fungi, such as Septoria (*spot disease*) and Heterosporium (*fairy ring*).

Very badly diseased plants, especially when much crowded and growing in damp atmosphere, have more yellowish green leaves than normal, of a more transparent appearance, and usually smaller. The lower leaves of diseased plants in any atmosphere or soil, die prematurely, and the vitality of the plant is so lowered as to check the growth and decrease the size and number of the flowers.

History of the investigation.—Before proceeding further it will be well to give some account of the course of discovery leading to the present understanding of the subject, which will among other things make clear a number of details of practical importance.

During the carnation season of 1887–8 the attention of the Station botanist was a number of times called to the abnormal deterioration of the plants, death of the leaves, failure of the flowers and general unhealthy appearance of some of the carnations grown by the University florist, Mr. Pierre Vanlandingham. This did not occur in all varieties alike, but was more conspicuous in some, especially in Buttercup. Mr. Vanlandingham considered the trouble due to some specific disease. Examination was made at different

*An obscure disease of carnations known as "purple joint" is not, so far as the authors know, in any way connected with bacteriosis.

times, but no parasites, either insect or fungous, could be detected even with the most careful microscopic exploration of both surface and interior tissues of the various organs of the plant.

In fact, no cause, either parasitic or non-parasitic, could be found to account for the difficulty. The eye, either unaided, or by the assistance of the microscope, detected only a gradual shriveling of the tissues of the leaves. At first light-colored blotches made their appearance, which gradually extended, coalesced, and finally the whole leaf became dry and lifeless.

After a time these dead leaves, while still clinging to the plant, became infested with various saprophytic fungi, more especially with *Volutella sp.* and *Cladosporium herbarum*. The plants in this stage presented a quite unsightly appearance to the florist's eye, and yielded few good flowers.

In January, 1889, an article by Mr. A. B. Seymour on the fungus, *Septoria Dianthi*, as a cause of carnation disease, appeared in the *American Florist*, and in again searching the plants in the University greenhouses (the Station not yet possessing any glass structures) for the possible occurrence of this malady, attention was first attracted to the semi-transparent dots in the green and unblotched leaves, already described. Repeated microscopic examinations of the sections through these spots convinced us that the cells of the region were always infested with bacteria, often in considerable numbers. It was not difficult to see that the conspicuous dry blotches might be derived from the inconspicuous dots, and that the latter possibly represented the beginnings of a genuine disease. There were now a number of difficult questions to answer, foremost of which was to show the causal or accidental relation of the bacteria seen in the cells to the degeneration of the tissues. The larger part of the work of the investigation from this time on until the main facts were established, a period of about eighteen months fell to the lot of Mr. Bolley.

Separation of the specific germ.—This proved to be a matter of considerable difficulty. Repeated trials for a number of months gave such varying results that nothing of a substantial nature was learned. Saprophytic forms from the air and from the leaves of the plant took possession of the cultures, obscuring or destroying the slower growing parasitic form. It was not until it occurred to us to acidify the culture media, in imitation of the acid plant juices, that the difficulties began to vanish. Malic acid was used; and the cultures were no longer crowded with extraneous forms, the saprophytic bacteria especially making little or no growth.

Thus we were enabled to separate a germ, very characteristic

in growth and appearance, which eventually proved to be the specific bearer of the disease.

Infection experiments.—At the same time that attempts were being made to separate a specific germ, various infection and inoculation tests were conducted. The contagium for these was for some time obtained by cutting up diseased leaves into nutrient fluid, at first of neutral and afterward acid reaction, and using the germ laden liquid, after permitting a day or two to intervene in which the bacteria might multiply.

The first successful transfer of the disease was made in March, 1889. On March 26 the interior tissues of a diseased leaf were cut out with a sterilized knife and dropped into potato infusion. On March 28 the infusion had become cloudy from the growth of bacteria, and a thin pellicle had formed. Application of the liquid was now made with a camel's hair brush to the surface of four young leaves of a small healthy carnation plant growing in the greenhouse. Indications of a change in the infected regions could be detected on April 12, and distinct characteristic dots where the infusion was applied were clearly seen on April 16, the remainder of the plant remaining quite free of any such appearance. (See fig. 2.)

Another plant was similarly treated with the same material on March 30, but with the added precaution to cover the plant with an open-topped belljar, plugged with cotton to guard against accidental infection or interference. Distinct evidences of the disease appeared on April 16. It was thus shown that the disease could be artificially transferred from one plant to another.

No pure cultures were yet available, and for various reasons, chiefly the lack of suitable plants and special appliances, the work was almost discontinued until fall.

In September, 1889, the work was again taken up, a greenhouse having been built in the meantime, and vigorously prosecuted. Numerous cultures were made from diseased leaves of the carnation, using both liquid and solid media. The liquid media were chiefly infusions of corn sprouts and of potato, mostly the latter, and the solid media were nutrient gelatin and agar-agar, variously prepared.

One of the most constant sorts obtained in the trials was a coccus-like form of a yellowish color, that grew slowly, but developed well in acid cultures. It was looked upon as the probable cause of the disease as early as October of this year, and was studied in various ways. Many surface infections were tried in the manner already described, but the purity of the cultures could not always be guaranteed, or else the trial plants developed diseased spots out-

side the inoculated area, and thus absolute certainty could not be attained.

January 15, 1890, an infection of three seedling carnations in the greenhouse, two protected with belljars and one uncovered, was made as previously described, using a potato infusion. This infusion had been infected the day before with the yellow coccus-like germs from a pure culture on solid media, which had originally been obtained from diseased carnation leaves on November 18, 1889. The affected areas began to show disease in six days, and in eleven days more all the leaves became "infected at the points of application, and at these points only," as stated in the record book, and remained so for a month and a half, or more (fig. 1). It was now believed that the germ causing the bacteriosis was found, and although many subsequent infection experiments were carried out with varying success, the remainder of the winter was chiefly given up to the biological study of the specific germ.

The best method for applying the contagium was for a time uncertain. The method used in studying pear blight, the well investigated bacterial disease of pomaceous trees, which consists in abrading the surface so that the germs may at once come in contact with the internal juices and tender tissues of the plant, proved inapplicable to the carnation disease. No clearly marked cases of the disease were obtained in this way. The wounds showed in some cases a slightly yellowish margin, but otherwise gave few indications of results differing from those which might arise from any accidental abrasions.

Surface application, wetting the uninjured surfaces of the young leaves with the germ-laden fluid, was finally adopted as the proper method of infection.

Experience showed that success could only rarely be attained when the infectious fluid was applied to mature leaves. The best results were always secured when the application was made to the small appressed leaves at the end of the stem. They were drawn back and well wet with the fluid, and if growing vigorously, usually showed the characteristic pellucid dots by the time the leaves were full size.

A difficulty was experienced in securing perfectly healthy plants, for it was found that nearly all carnation plants, whether grown in the greenhouse or out of doors, in pots or in beds, showed more or less evident traces of the disease, when examined critically.

Description of the germ.—The microbes, as they appear in the cells of the host, are oval or elliptical in outline, not united into chains or filaments, but almost or quite separate from one another,

and about one and one half times as long as broad (figs. 11 and 20). They are of medium size (.9–1.25×1–2μ), being neither among the largest nor the smallest of bacteria. Within the tissues of the host they do not exhibit independent movement, and undergo no marked variations in form during growth.

When transferred to richly nutritive media, which are sufficiently moist, they undergo rapid division, often forming filaments of considerable length (15–300μ), are highly motile, and finally pass into a zoogloœa state of rest (figs. 12 and 15). In old cultures on solid media they present about the same individual appearance as in living tissues of the carnation (fig. 11).

In mass these bacteria have a yellow color, which is more strongly developed in presence of free acid. The color accumulates slowly as the bacteria grow (figs. 3 to 5), and is apparently not deposited within the cell, but is an excretion from it.

No spores have been found in any stage of development or under any conditions of growth, and it appears to be strictly an arthrosporous form.

Although it grows readily in artificial cultures it has only been found in nature in leaves of the carnation pink (a horticultural form of *Dianthus Caryophyllus*). Infection experiments have shown that it can be transferred to the clove pink (*D. Caryophyllus*), Pheasant's-eye pink (*D. plumarius*), Japan pink (*D. Japonicus*), Indian pink (*D. Chinensis*), and Sweet William (*D. barbatus*), but not to the shoots, leaves or tubers of potato, or to other non-caryophyllaceous plants.

From these facts it is concluded that the germ of the disease is parasitic only upon pinks, and is a new and undescribed form. It is therefore proposed to name it *Bacterium Dianthi*.*

Isolation of the germ.—Plate cultures of nutrient acid gelatin were used for this purpose. The gelatin may be made so strongly acid that, so far as we are aware, no atmospheric or ordinary saprophytic forms will materially interfere with the development of the germ. Malic acid was used, but doubtless other kinds will accomplish the same purpose.

A number of ways for transferring the germs from the leaf to the gelatin were tried, all more or less uncertain. The chief difficulty was due to the simultaneous transference of other germs,

**Bacterium Dianthi* Arth. and Boll. *n. sp.*—Cells oval to elliptical, single or rarely united, .9-1.25×1.-2. u: in rich fluid media more united, in part forming short filaments, at first motile, afterward forming distinct elongated, somewhat convoluted, zoogloœa; on solid media becoming yellow in mass.

Habitat: Mesophyll cells in the leaves of carnation pink.

which occur on the surface of the leaf, or drop in from the air, and which grow so much faster in the cultures as to overrun the plates before the form sought can be seen. As just stated, acidifying the medium is a partial and serviceable corrective.

In all cases it is best to select leaves in which the disease shows as small dots when examined by transmitted light, but gives no surface evidence of its presence. In such leaves, the younger the better, the disease is not so far advanced as to cause breaking down of the tissues, which would give a footing for other confusing forms of bacteria.

At first the outer surface of the leaf selected was removed with a flamed knife, and the inner portion cut up and dropped into the nutrient medium. Although the leaf is somewhat fleshy, yet it is too small, and the infected spots are too minute and too near the surface to make this a convenient method.

Attempts to free the surface of the leaf from other germs by washing with corrosive sublimate (one pro mille solution of bichloride of mercury) always gave negative results. Some of the poison passed over into the culture and prevented growth, even when the leaf was well washed with distilled water after its treatment with the corrosive sublimate.

Most of the work was done with cultures obtained by passing the leaf quickly through a Bunsen gas flame two or three times, thus destroying all surface molds and bacteria, in both spore and vegetative condition, and then cutting the leaf into thin sections with flamed scissors, allowing the sections to drop into the nutrient medium.

Either the fragments of diseased leaf were put directly into liquefied gelatin, which was shaken and then spread out over a glass plate, or more frequently they were dropped into a vegetable bouillon, and after a day or two a minute quantity of the germ-filled bouillon was transferred to liquefied gelatin and then to a plate. In either case the colonies which shortly appeared upon the plate were transferred to test tubes, and pure cultures thus obtained.

Plate cultures of the germ.—The method of preparation is to inoculate a tube of 10% acid, nutrient gelatin, made liquid by warming. The inoculation is performed by inserting a germ-laden platinum needle into the gelatin, the germs having been secured by dipping the needle into a liquid (or solid) culture of the germ. The tube is then shaken up and flowed evenly over sterilized plates (or Petri dishes may be used), which are kept in a moist chamber in a cool place, so that the gelatin readily sets in a thin film over the glass. Dilution methods may be introduced if deemed necessary.

If the work has been well done so that the bacteria were thoroughly separated before the gelatin was poured over the plate, each individual germ, beginning to multiply very rapidly, forms in the space of two or three days numerous separate specks in the nutrient gelatin, each representing a colony which has arisen by multiplication from the scattered individuals in the culture. The appearance of the colonies of *Bacterium Dianthi* is quite characteristic; and if other bacteria grow upon the plate, microscopic characters permit of discrimination to a considerable degree, as a rule.

In neutral plate cultures of *Bacterium Dianthi* the colonies after a time usually consist of a few light colored zoogloea bodies with a surrounding irregular area of actively growing bacteria, the whole having a light cream color (fig. 13). These colonies spread irregularly, and coalescing, form what appear to the unaided eye as comparatively large viscid drops.

In acid plate cultures the zoogloea make up nearly the entire body of the colony, giving a more irregular outline and a lobed and wrinkled appearance to the surface (fig. 14). The color is a light clear orange. When growth is strong the colonies pile up into pronounced yellow viscid drops.

Test tube cultures with solid media.—Streak cultures on gelatin*, made by dragging a germ-laden needle along the surface of the medium, at first show a smooth even growth along the track of the needle, and a pale cream color. At this stage the bacteria are very motile and are in the most active period of division (fig. 3). After a few days little viscous elevations gather at various points along the margins and over the surface, giving the culture a somewhat mottled appearance, while the color deepens to a bright orange, especially along the middle line (fig. 4), the color being much deeper in acid cultures.

The germs possess an activity which causes individual bacteria to penetrate the gelatin, and to become the centers of colonies at various depths below the surface. This gives a peculiar feathered appearance, the dots seeming to radiate from the needle track (fig. 5). Acid cultures, being more favorable to growth, are also more favorable to this feathering. The solidity of the medium, however, exercises the chief control. With 90 % or more of water the bacteria

*Gelatin medium is made by using 1500 cc. water in which 1½ lbs. of minced fresh meat have soaked for a day, adding 100 g. gelatin, 3 to 5 g. table salt, 10–12 g. pepsin, and heating (preferably in steam sterilizer) until the gelatin is fully liquefied. Neutralize with sodium carbonate, or acidulate with malic acid, as neutral or acid media may be desired. If neither is done the culture material will be slightly acid. Finally clarify with white of egg.

penetrate the medium readily, but with 85 % or less there is little or no penetration.

Liquefaction of the gelatin takes place slowly, not becoming noticeable until eight or ten days in room temperature. Complete liquefaction will eventually take place.

Stab cultures, made by thrusting a germ-laden needle into the middle of a tube of nutrient gelatin, show but little growth or color along the track of the needle, but an abundant feathering of the germs in all directions. There is no accumulation of gas, and probably none is formed.

On agar-agar* the appearance of the cultures is not materially different from that on gelatin, except that the germs do not penetrate the medium, and no feathering takes place. The granulation of the surface of the culture is usually less pronounced, and as would be expected, no liquefaction occurs.

On slices of cooked potato the growth is abundant, and similar to that on agar-agar.

Test tube cultures with liquid media.—The liquid media used in these studies were infusions of corn (maize) seedlings† and of potato tubers.‡ In either of these media, especially when slightly acid, the germs make a luxuriant growth. The liquid becomes turbid after some hours, due to the rapid multiplication of the bacteria, and in one or two days a pellicle forms over the surface. The pellicle has a granular or finely mottled appearance. Both the pellicle and the turbid mass beneath are at first whitish, becoming pale cream color with age.

Imbedded in the pellicle and floating free in the liquid are zoogloea bodies in great abundance. In about two weeks the pellicle begins to disintegrate, and a precipitate gathers at the bottom of the tube.

Drop cultures under the microscope.—By growing the bacteria in a

*The culture medium is made in essentially the same way as the gelatin medium, except that 15 g. of agar-agar are substituted for the 100 g. of gelatin.

†To make corn infusion cut up finely a quantity of corn seedlings, germinated between folds of moist cotton, having the shoots an inch or two long, and boil for a while in water, or until the liquid becomes red. Cool, and add some pepsin, boil again and finally filter. It will be slightly acid. If wanted neutral or more strongly acid, it should be made so before the last boiling. This makes a very transparent liquid, free from sediment, although usually highly colored.

‡To make potato infusion slice some pared potatoes into enough water to fully cover them, and heat without boiling for some hours. Pour off the liquid, partly cool, add white of egg, heat again for an hour in steam sterilizer, and after thoroughly settling again decant the liquid. Add a small amount of malic acid and some pepsin, heat in steam sterilizer one half hour, then filter. To obtain a neutral solution neutralize and heat again just before the last filtering. This process should give a perfectly transparent and colorless liquid, which will remain free from sediment.

drop of nutrient fluid in a moist chamber upon a microscope slide the continuous observation of the growth and development of the germs is made possible. In such a preparation the changes of form due to growth, all stages of division in the production of new individuals, and the formation and final disintegration of zoogloea may be readily traced.

It is in this way that the filaments, occurring in fluid cultures undergoing rapid growth, are seen to arise from multiplying bacteria, in which the new cells tardily separate from one another. Such filaments display more or less indication of constrictions and cross walls, here and there (fig. 12 *c*), and readily fall apart when the liquid is agitated.

Individuals in very rapid growth are normally motile. The movement is like that of a flagellated organism, but no cilia have been seen. Whether they occur or not can not be stated, as no careful examination for them has been attempted. Motile cells (fig. 12 *a*) exist not only singly, but even more commonly in pairs; and often two or more pairs may remain united, until the filament becomes too unwieldy for movement. The parts of a filament and especially two pairs of cells, may be held together by a kind of flexible joint, which suggests the presence of a connecting flagellum, as known in other species of bacteria.

The production of zoogloea is particularly interesting. Certain individuals, without ceasing active multiplication, become nonmotile, and at once begin to excrete a gelatinous envelope (fig. 12 *b*). This envelope offers considerable resistance to longitudinal extension, and the new cells as they form slip past one another, accumulating in an elongated mass, which increases faster in thickness than in length (fig. 12 *d*). The mechanical resistance offered by the gelatinous envelope has undoubtedly much to do with the rounded, lobulated outline of mature zoogloea (fig. 15). If the nutrient fluid is not renewed, the zoogloea disintegrate in ten to fifteen days by liquefaction of the gelatinous envelope, permitting the bacteria to fall to the bottom of the fluid as independent inert cells.

The rate of multiplication also can be determined in a hanging drop, as it is possible to continuously observe the same individual for some time. Two individuals were thus watched through a full generation, and two others for a somewhat longer period, (figs. 6, 7, 8, 9 and 10), the temperature being about 35° C. It was found that a bacterium of the typical form (fig. 6, 2 and 4) showed a well marked constriction or even a cross wall within seven minutes, (fig. 7, 2′ and 4′) and in twenty minutes more there were two full grown bacteria formed from each original cell (fig. 8, 2″ and 4″), although

still attached to each other. Each of these cells may divide, and a chain of four cells result (fig. 9, 4'''), or the cells may separate and the process be repeated (figs. 6, 7, 8, 9 and 10, 3).

We may conclude from these observations that under good conditions for growth an individual of *Bacterium Dianthi* may become two within a half hour, and these two increase to four in the second half hour, and so on. At this rate there would be sixteen at the end of two hours, sixty-four at the end of three hours, 256 at the end of four hours, over sixteen millions at the end of twelve hours, and over 280 billions at the end of one day. Is it any wonder that a few germs placed in a test tube culture will make it turbid within twenty-four hours? Although the individual bacteria are very minute, requiring over 1500 of them placed side by side to extend the sixteenth of an inch, yet the 280 billions that may be formed from one germ within a day represent no insignificant bulk. They would in fact occupy fully a cubic inch of space. As it takes the material of three or four fluid cultures to fill a cubic inch, it is evident that even under the favorable condition of artificial cultivation the food supply soon becomes inadequate to keep up a maximun rate of growth.

Action of gases with varying tension.—To secure pressure a fresh test tube culture on neutral agar-agar was attached directly to a small gas generator (Kipp's pattern), remaining connected under a pressure of about 25 cm. of water for five days.

In carbon dioxide under pressure a very slight growth occurred without coloration, and the original inoculated germs lost what color they first possessed. The slight growth may be assumed to be due to a small amount of oxygen remaining in the agar-agar substratum, which could not be readily displaced by the method pursued.

In hydrogen under pressure the growth was almost normal in amount but with very faint coloration.

In atmospheric air under pressure the growth was to all appearance normal in both amount and color.

A partial vacuum was secured by first partially drawing out the upper portion of the tube containing the culture, by holding over a flame, then attaching the tube to a water pump. When the exhaustion was carried as far as possible, the constriction and sealing of the tube was completed in a flame.

Cultures on neutral agar-agar were sealed when the vacuum pump registered 730 mm., the barometer standing at 742 mm. In the first twenty-four hours a slight growth occurred, due, it may be supposed, to the small amount of air still confined in the tube, both

without and within the substratum. No further change took place. After twenty-five days air was admitted to the tubes, and a normal growth at once sprung up, with the usual coloration.

It is apparent from these tests that *Bacterium Dianthi* is an aerobic germ, requiring free oxygen for its development, and that the development of color also depends upon the presence of free oxygen.

Effects of temperature upon growth.—The range of temperature for the activity of the germs is very wide. They make comparatively rapid growth at 8 10° C., which is the temperature of the chamber of the refrigerator used in the laboratory. It is therefore presumable that it would take a very much lower temperature to stop their growth, and that they might even be subjected to freezing without permanent injury, as is the case with many species of bacteria.

The upper ranges of temperature were more carefully tested. A drop culture was used, and the time required for a cell to divide and become two was taken as an index of the effect of the heat. To secure a uniform temperature the microscope with the culture slide in place was put inside a Pfeiffer warm chamber, which was provided with a thermo-regulator, and otherwise arranged to give perfect control of the heat. It was found that the rate of division increased up to 34–36° C. (93–97° F.), but as the temperature rose above this point the division was less rapid. The highest temperature used was 45° C. (113° F.), which still gave a fair rate of growth and cell division, showing that the point at which growth would entirely cease must be relatively high.

Staining the germs.—In regard to this mode of study and differentiation nothing of characteristic value was noted other than that in all stages of its development the bacterium stains readily with most of the dyes in general use, the ones most successfully used being aqueous solution of fuchsin and of gentian-violet.

The germ as a parasite.—Sections through diseased carnation leaves in the earlier stages of bacteriosis reveal the individual bacteria for the most part imbedded in the protoplasm, with only a few here and there in the cell sap occupying the vacuoles. There are often a score or two in each cell, but they are rarely numerous enough, even in the later stages of the disease, to form the chief part of the cell contents.

The first marked disturbance in the cell is the gradual disorganization of the chlorophyll granules. This is followed by the breaking up of the protoplasmic utricle, and the general disintegration

of the whole cell contents. The osmotic action of the cell is early interfered with, at first making the diseased tissue more transparent, and afterward permitting it to dry out and collapse, forming dead spots in the leaf.

The bacteria progress from cell to cell very slowly, and each diseased area is long kept within narrow and well circumscribed limits. The danger from the germ as a parasite lies rather in the great number of chances for centers of infection than in its aggressive character.

That the conditions of its life within the carnation plant are not the best for its highest development is evident enough. In the first place there is no apparent movement; and the evidence of direct observation would mislead one into believing it to be a non-motile organism. But there must in reality be a slow movement of some sort, as it has the power to travel from cell to cell. It may be assumed that it has independent motion within the plant, in kind like that which is so conspicuous a feature of its development under artificial conditions, but so reduced as to be too slow to be detected by the usual methods of microscopic observation.

Again it is noticeable that in the plant the cells are rarely united, even in pairs, and never in filaments. Their form and appearance is that of growth in old artificial cultures, from which the nutriment has been largely extracted (compare figs. 11, 12 and 20).

We may therefore safely conclude that although *Bacterium Dianthi* is a genuine parasite, inhabiting only members of the pink family, as infection experiments already referred to indicate, yet it can also exist outside of living plants, probably in natural infusions of decomposing vegetation. Hence it may be considered a facultative saprophyte.

Mode of penetrating cell walls.—How parasitic bacteria make their way from one cell to another is a question of some interest. It is difficult or impossible to find perforations in the cell wall through which they might have passed; and yet they can be found in the interior tissues, imbedded in the cell contents, and no theory can fully account for their presence except the one that they have perforated and passed bodily through the wall.

The matter attracted the attention of the first person who carefully studied a bacterial disease of plants. Burrill* in his account of pear blight says, "Unless the bacterium does dissolve for itself a passageway through the cell wall, it cannot possibly gain entrance

*"Anthrax of fruit trees," Proc. A. A. A. S., xxix (1880), p. 594; "Blight of pear and apple trees," Trans. Ill. Univ., 1880, pp. 74-75.

in the adult state, yet they are found swarming in cells absolutely closed so far as a power of one thousand diameters can reveal." His speculations left the subject without indicating with any certainty the direction in which the solution of the problem lay.

At the present time we feel certain that the organism does "dissolve for itself a passageway," even if the opening cannot afterward be detected. It was shown by Marshall Ward* in 1888 that parasitic fungi excrete an enzym, by which they dissolve the cell wall against which the ends of the hyphæ press. Since then numerous investigations have demonstrated the excretion of enzyms by different bacteria as well as fungi, and we cannot doubt that it is in this manner that the bacteria of pear blight and carnation bacteriosis make their way from cell to cell.

Although under ordinary circumstances no openings can be found in the wall to show where the bacteria passed through, yet this is not always true. The disease as seen in the Japan pink through artificial infection, as well as in the other varieties having thin yellow-green leaves, exhibited many instances of the corrosion of the walls by the bacteria. The cell walls of such pinks are much thinner and more delicate than in the varieties with narrow, fleshy, pea-green leaves, and are more readily affected by the parasite. When the tissues are heavily infested with the germs, openings of considerable size may often be seen in the walls, apparently due to the cumulative erosive action of the bacteria (fig. 20). In some instances the germs are found appressed to the wall, which is visibly eroded in places, so that the parasite may be sunken half its depth into the wall, the complete perforation being only a question of time. There is an evident connection between the ready solubility of the walls and the virulence of the disease in these several varieties of pinks.

In those cases in which bacteria are found in the cells and no entrance visible, we may believe that they have dissolved a passage through the wall by action of an enzym, and that the preforation is either quickly healed by growth and swelling of the wall, or that it is veiled by the layer of protoplasm lining the cell, of which alternatives the first is much the more probable.

Entrance of germs into the host.—Our study of the disease gives no evidence that the germs are able to effect an entrance through the cuticle of the host by means of an enzymic perforation. On the contrary, they are dependent upon either natural openings (stomata) or accidental punctures (insect stings) to get inside the plant.

*"A lily disease," *Annals of Botany*, ii (1888), pp. 337-356.

The stomata (breathing pores) are the usual points of attack. Germs of the disease which have been deposited upon the surface of the host by wind or rain find their way through the opening between the guard cells into the air chamber beneath. Here they attach themselves to the walls of the surrounding parenchyma cells, and dissolving a passage gain entrance to the living tissues of the leaf (figs. 19 and 20). In artificial infection, using a large number of vigorous germs, it was found that with thin-leaved pinks, not only the cellulose walls of the inner tissues, but the cuticularized outer walls of the epidermis were often broken down, apparently the attack coming from within (fig. 20 *c*, *c*). However, this very rarely or never occurs in the fleshy leaved varieties, and in any case would be especially rare under natural conditions.

It is evident that after the bacteria fall upon the leaf sufficient moisture must be present so they may move about and find their way into the cavities of the stomata. A natural dew or artificial sprinkling will furnish the needed moisture. And so it is that plants in a greenhouse where the air is kept moisture laden will show most disease.

In both natural and artificial infection the earliest indication of the disease is the appearance of translucent dots, which are found by microscopic examination to be always underneath stomata. These dots are scattered over the leaf (figs. 1, 2 and 17), and are round until they coalesce with adjoining dots, and thus form dots or areas of irregular outline (fig. 18). It may be inferred from this that natural infection is usually through the stomatal openings.

It was early found that it is difficult to introduce the disease into mature leaves, but that it readily attacks young and partly grown leaves. From what has been stated it is not difficult to give an explanation of this apparent preference. The cellulose walls of the stomatal air cavities are easily penetrated in young leaves, but in mature leaves they have become incrusted with lime salts deposited from the evaporating water, and are also somewhat changed chemically, making them less susceptible to the action of enzym, and consequently almost impervious to the bacteria.

The question might be asked why other species of bacteria do not gain entrance to the tissues in this manner also, for it is a fact that the surface of the carnation leaf, and practically of all foliage, is infested with numbers of bacteria, and often of yeasts. In fact, in studying the disease one of the most troublesome intruders into the cultures was *Bacillus subtilis* commonly called hay bacteria, which seemed to be a constant inhabitant of the leaf surface. Other species appeared less regularly. The

first reason for immunity against these surface and other bacteria is doubtless the acid reaction of the plant juices, for we have seen that in artificial cultures, acidifying the medium hindered or prevented the growth of other forms. The bacteria which find their way into the stomatal chambers, therefore, develop no further on account of the acid sap. The resistance of the living protoplasm is doubtless another strong factor, preventing most germs from making any advances, even after gaining an entrance to the interior of the cell. And it may be that the secretion of a proper enzym is lacking, although on this point we have no evidence.

When the disease has progressed sufficiently to kill the cells, saprophytic bacteria, and even fungi, are not slow to take possession and complete the work of destruction (fig. 19). This accounts for the fact that the lower leaves of a diseased plant often rot or mold far worse than of a healthy plant under same conditions.

Beside gaining entrance to the plant through natural openings the germs of bacteriosis may enter through insect punctures. The only insects that require attention in this connection are plant lice (aphides). The common green fly, or aphis, of the greenhouse may in some instances prove such an efficient bearer of the contagion that every leaf on a plant may be inoculated at hundreds of points, and the whole plant be turned a sickly yellow by the growth of the bacteria in the tissues. It is evident that only in a neglected greenhouse will the disease be seriously spread by this means, for only in such would the aphis be allowed to flourish.

Varieties of carnation affected.—No varieties of the carnation are exempt from the disease, but they differ much in susceptibility. The seat of this difference is chiefly in the vigor of the plant. Weakly varieties like Buttercup, Sunrise and La Purite', are most affected, while seedlings are least affected. Poorly grown plants are more affected than those well grown. Partly starved or stunted plants are specially liable to attack. It must be remembered in this connection, however, that plants of any degree of vigor may be essentially free from the disease because the foliage is kept dry, so that the germs can not pass from the surface into the stomata, and aphides are absent. Susceptibility can only be shown where the conditions for the spread of the disease are present. But, on the other hand, if the conditions are particularly favorable to the disease, any plant, however vigorous, may succumb.

Distribution of the disease.—It is not at present possible to state the extent to which the disease is distributed, owing to the fact that florists have not yet become sufficiently familiar with it as a distinct

disease, and so there are no published data. The writers have seen it in greenhouses at the following localities, or have seen cuttings from the places, showing the disease: Indianapolis, LaFayette and many other places in Indiana, Buffalo, Boston, New York, Toronto (Can.), Chicago and Lincoln (Neb.). From the examination of much material, and from the appearance of the disease in the several localities where personal examination was made, it is safe to say that it is common throughout eastern North America, and furthermore the indications are that no locality in this wide region, where any considerable number of carnation plants are grown, is entirely free from it. One may say that a marked characteristic of the disease is its universality.

Economic aspect of the disease.—The bearing upon carnation culture from the commercial standpoint is a matter of moment. It needs no argument to demonstrate that any considerable attack of parasitic bacteria as in the case of any other form of parasite, will weaken the plant and lessen the harvest which the plant would naturally yield, and carried to the extreme will render the plant worthless or even kill it.

It is in accordance with the writers' observations that, while bacteriosis of carnations rarely kills a plant, it does frequently reduce the strength of the plant to a degree decidedly unprofitable to the florist. Even when casual observation sees nothing wrong with the plants, there may be enough disease present in the foliage to decidedly lessen the yield of flowers, or to injure the growth of cuttings.

The grower of cut flowers especially must guard against the inroads of this disease, if he would have the fullest returns in the way of both quantity and quality of bloom. It is the insidious nature of the disease which makes it so especially formidable. It establishes itself among the florist's plants, flourishes and takes full possession of the bench without being detected. The result is that the flowers do not reach their maximum size, and are far less in number than the variety, soil and culture would warrant. Thus the legitimate profits of the business are reduced.

Mr. Fred. Dorner, well known for his eminent success in growing fine carnations, said in a recent paper before the Society of American Florists* that "of all diseases [of the carnation] the bacteria and rust are the two most prevalent and most dangerous on account of the epidemic nature of the former and the wide spread dissemination of the latter." He gives at considerable length the

*American Florist, xi (1895), p. 87.

conditions under which he has observed the bacteriosis to flourish. "We mostly find some sporadic cases in every establishment," he says, "telling us of their presence, and when these warnings are neglected they may become epidemic."

There can be no full return of profit in plant culture without healthy foliage. To let the lower leaves of the carnation plants die, when they might as well be alive and nourishing the plant, is or the commercial florist to throw away a part of his available income, and is very impolitic for the amateur. The uncontrolled presence of the disease will lead to this. To permit the disease to have any hold whatever upon the plants such as might be readily avoided, is a voluntary relinquishment of a portion of one's legitimate profits, where the carnation is grown for commercial purposes.

Methods for controlling the disease.—The knowledge that has been gained by studying the cause and nature of bacteriosis makes clear a rational means of controlling it. As soon as the way in which the germs found entrance into the plant was discovered, a method of keeping them out presented itself.

As there must be moisture upon the leaves sufficient to enable the bacteria to move about and enter the stomata, in order that they may gain access to the interior of the leaf, it is evident that keeping the foliage dry will prevent the disease. Putting this inference to a practical test proves it to be well founded. In applying the preventive upon a commercial scale difficulties are at once encountered, chief of which are the advent of the red spider when foliage is kept dry, and the inconvenience of keeping the soil wet without wetting the foliage. But these difficulties can be adjusted.

The development of a rational method of treating the carnation was hastened by the increasing interest among florists in other carnation diseases, and especially in carnation rust, which first attracted attention in this country in 1891. This and other fungous diseases are much promoted by the presence of surface moisture; and a sparing use of water on the foliage is advocated in order to keep them severally in check. In the discussion which followed the reading of a paper on rust before the American Carnation Society at Buffalo, in 1892, it was apparent that a majority of the growers present believed that, if anything was to be gained by it, there would be no great difficulty in so watering a carnation house that the foliage could be kept practically dry and yet free from red spider. Although there was much diversity of opinion regarding details, yet the essential features were (1) to sprinkle from overhead at long intervals, say once a week in spring and fall, and less frequently in winter,

and only on bright warm days when there would be rapid evaporation, thus giving sufficient moisture for the foliage, and (2) to wet the soil by sending a stream of water along the surface of the ground between the rows, thus watering the roots without wetting the leaves. At no time should the plants be watered overhead, or sprayed, at such a time that the foliage will remain wet over night.

An improvement upon this method would be to do all watering between the rows beneath the foliage, and to spray the foliage as much as necessity may require with a weak fungicide, such as one of the copper compounds. Bordeaux mixture may be used, or ammoniacal copper carbonate.* The latter is to be preferred, as it does not stain the foliage.

Keeping the foliage dry is in direct line with the natural requirements of the plant. The carnation shows by its structure that it is adapted to a dry atmosphere. The thick smooth leaves, the firm epidermis and the waxy bloom that whitens the surface, are all devices for conserving moisture. A fairly moist soil and a dry atmosphere are, therefore, the ideal requirements for the carnation, as indicated by the structure of the plant; and the experience of the observant florist corroborates the deduction.

Some of the progressive commercial carnationists have devised methods of treating the carnation under glass, that leaves little to be desired in connection with the subject of bacteriosis. Mr. Fred. Dorner early adopted what has become by slight subsequent modifications a satisfactory system for guarding against bacteriosis. A similar system was also early put into use by Mr. C. W. Ward, also a very successful carnationist.

Mr. Dorner† uses netting of galvanized iron wire with about one inch mesh cut into strips about fifteen inches wide. These strips are bent into a somewhat rounded /\-form, about six inches high and eight inches wide, and placed between the rows of plants (fig. 22). A support for the foliage is thus made, which gives perfect circulation of air beneath, and permits of watering the soil without wetting the leaves. The flowering stems are supported upright by a network of twine stayed by wires, stretched above at two different levels (figs. 21 and 22).

Mr. Dorner has authorized the following statement of his method

*The ammoniacal copper carbonate is prepared by adding enough water to five ounces of copper carbonate in a wooden pail to make a thick paste. Then add enough ammonia (about three pints) to fully dissolve the copper. This may be kept in a tightly stoppered bottle as a stock solution, and a little added to the water at each sprinkling. It should be diluted about one hundred times, that is the formula will make about fifty gallons of an ordinary strength fungicide.

†*American Florist*, x (1894) p. 196.

of watering, which is the most important part of the treatment: The beds are watered in the usual way in the early fall, while the plants are yet so small that the foliage only partially shades the ground, and the weather does not necessitate the use of furnace heat. After becoming well established the wire netting is put in place between the rows, first removing all dead leaves and useless branches from the plants. The watering is now done entirely by placing the end of the hose between the rows under the wire screen. The usual nozzle is discarded, as the stream of water is too strong, and washes the soil away from the roots, and in its place a special form of nozzle is used, devised by Mr. Dorner. This discharges the water laterally through quarter inch openings, the force of the stream having been broken by striking against the closed end formed of an inturned cone of metal. The amount of the watering is a matter of judgment, and can not be stated in the form of a rule. It depends very largely upon the weather outside, and also upon a variety of other conditions. The evaporation from the benches and walks is sufficient to keep the air of the house moist enough for the best health of the plants, and spraying the foliage is only rarely done after firing begins. During the winter of 1894-95 the foliage was not sprayed from November until April, and a thoroughly healthy growth was maintained, with an almost entire absence of red spider, less than a half dozen plants showing any trace of the insect. In previous years it has been the custom to spray the foliage at intervals of about three weeks with water, to which a small amount of copper sulphate and ammonia have been added, making a very weak fungicide. The quantities used were not reduced to any fixed amounts. The spraying was only done on bright days, when the plants would dry off before night. It is believed that not a little of the success of this treatment is due to the vigilance with which it is carried out. Much care is taken that only healthy plants are placed in the houses, and constant watchfulness is exercised that by no accident or oversight does the disease gain a foothold. The aim is to have healthy plants, and to keep them so by guarding every avenue of attack.

There is similarity to Mr. Dorner's method, and yet some important differences in the details as arranged by Mr. C. W. Ward,* who also uses a wire netting, but bent into a sharp /\-shape. He uses, after having tried and discarded various other kinds, a half inch square meshed net called wire lathing, and finds that it is easier handled and lasts longer than the twisted mesh net. It is bought in 150-foot

Florists' Exchange, vii (1894), p. 83.

rolls, the widths used being twelve inches for dwarf growers and fifteen inches for tall growers. It is cut into lengths about four inches shorter than the inside width of the bench, and doubled over a board to the required /\-shape, and is then shoved in between the rows(fig. 24). A system of wires and strings above, somewhat similar to Mr. Dorner's, supports the flowering stems (fig. 23*).

The advantages of this plan are, according to Mr. Ward: "A sufficient support to base of plant to keep it off the ground and afford ample circulation of air through the open space to keep the soil properly ventilated. This space affords first-class facilities for cultivating the soil, watering without wetting the foliage, as well as spraying the base of the plants and applying any needed mulch, while the flower stems are erectly supported in a free, airy and natural position, without bunching and without any shading of the plants, and blooms can be neatly and rapidly picked."

Both the teaching of the laboratory and the experience of the cultivator unite in showing that an efficient protection against bacteriosis of carnations under glass (the disease is not troublesome out of doors) is to support the foliage well above the ground so as to permit free circulation of air, water the soil without wetting the foliage, spray the foliage at suitable intervals with a weak fungicide, only spray when the foliage will dry rapidly, ventilate well to prevent the air from becoming too moist, and keep the plants free from green aphis. The methods for carrying out this program, which have already been given, can be modified to meet the needs of individual carnationists.

It is well to note that this line of treatment is equally well adapted to prevent rust, spot, fairy ring, and the other fungous diseases which attack the carnation plant.

SUMMARY.

1. Bacteriosis of carnations is a widespread disease of the carnation plant only recently recognized.

2. It is seated in the leaves, but affects the whole growth of the plant, and checks the production of flowers.

3. The disease is caused by parasitic bacteria entering the plant from the air through the stomata, or occasionally by the punctures of aphides.

4. The germ associated with the disease may be separated

*For the use of figs. 23 and 24 the Station is indebted to the kindness of the publishers of the *Florists' Exchange*.

and shown by artificial infection of healthy plants to be the cause of the disease.

5. The germ will grow well at any temperature not inimical to the life of the carnation plant.

6. The disease does not usually affect other pinks beside the carnation, but may be artificially transferred to several species.

7. Plants outside the pink family will not contract the disease, naturally or artificially.

8. Any varieties of carnation may be affected, but old and weak or poorly grown varieties are most susceptible.

9. Plants may be kept essentially free from the disease by keeping the foliage dry and preventing the presence of aphides.

10. Watering a carnation house is to be done by directing the stream of water between the rows beneath wire netting arranged to support the foliage.

11. Overhead spraying is only to be done occasionally on bright days, and with water containing a small amount of ammoniacal copper carbonate.

EXPLANATION OF PLATES.

Plate I. - Fig. 1. Branch of carnation showing diseased areas at *a*, *b* and *c*. Plant inoculated from pure culture in potato infusion on Jan. 15, 1890. Infectious material applied to surface of youngest expanded leaves at end of stem. Drawing made after six weeks, showing the small yellowish dots of the disease where the infection was applied, and all other parts of the plant normally healthy. The leaf marked *c* was so small at the time of inoculation that only its tip was touched by the fluid, while the four or five leaves beyond this were too far within the bud to be reached by the fluid, and entirely escaped the disease. Natural size and color.

Fig. 2. Single leaf of carnation, upper surface, showing yellowish dots where bacteriosis has started from a surface application of fluid culture, made March 28, 1889. Area of application discolored by culture fluid. Natural size and color.

Plate II.—Test tube cultures of *Bacterium Dianthi* on nutrient acid gelatin, to show characteristic appearance. Temperature 12° C. Drawn natural size and color.

Fig. 3. Three days' growth. Yellow color not yet noticeably developed.

Fig. 4. Ten days' growth. Showing the characteristic yellow color and special outline of growth.

Fig. 5. Same culture as shown in Fig. 4, but turned one-fourth around to show the peculiar, "feathered" manner in which the germs have penetrated the gelatin and formed colonies beneath the surface. This only occurs when the gelatin is of a certain degree of softness.

Plate III.—*Bacterium Dianthi:* Figs. 6-10 show the changes in form and rate of multiplication of four individuals under continuous observation. Temperature 35° C maintained by a thermo-regulator.

Fig. 6. The four individuals at the beginning of the period.

Fig. 7. The four individuals after 7 minutes.

Fig. 8. After 27 minutes.

Fig. 9. After 32 minutes; two of the individuals moved outside of the field of the microscope, and could not be readily followed.

Fig. 10. After 39 minutes; only one of the original series of individuals remains within the field of vision.

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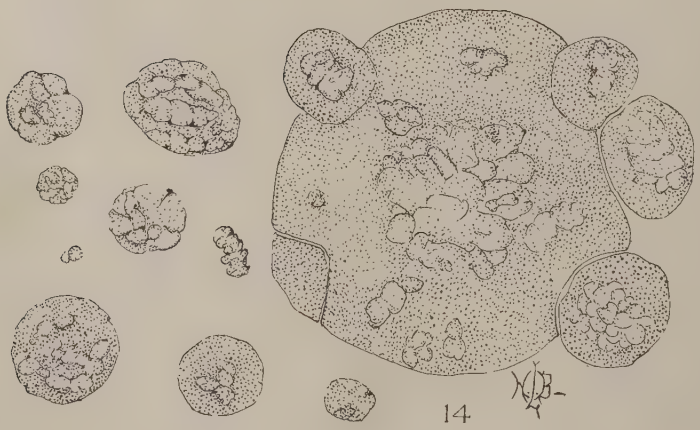
CARNATION LEAVES AFFECTED WITH BACTERIOSIS.

THE HELIOTYPE PRINTING CO., BOSTON

Fig. 3. Fig 4 Fig 5

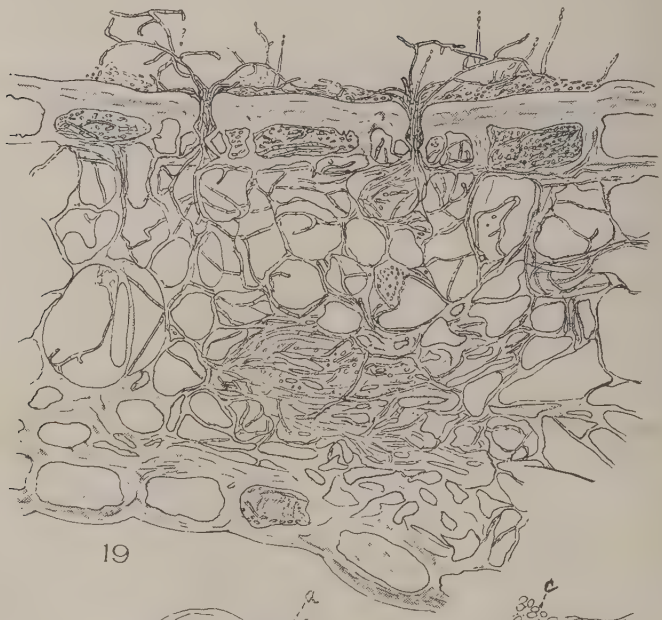
CULTURE OF BACTERIUM DIANTHI ON GELATINE

BACTERIUM DIANTHI IN VARIOUS STAGES OF GROWTH.



COLONIES OF BACTERIUM DIANTHI.

GROSS APREARANCE OF HEALTHY AND DISEASED LEAVES.
ZOOGLŒA IN DROP CULTURE.



SECTIONS OF BACTERIOSED LEAVES IN BOTH EARLIER (20) AND LATER (19) STAGES OF DISEASE.

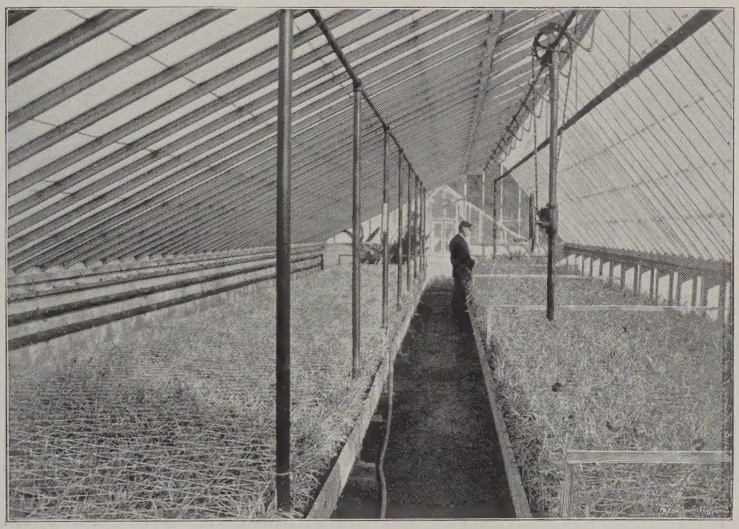


Fig. 21.

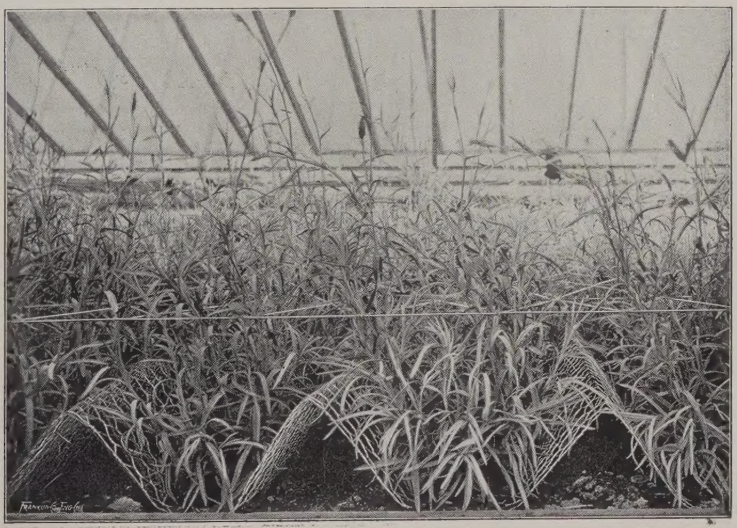


Fig. 22.

VIEWS IN MR. DORNER'S CARNATION HOUSES.

Fig. 23.

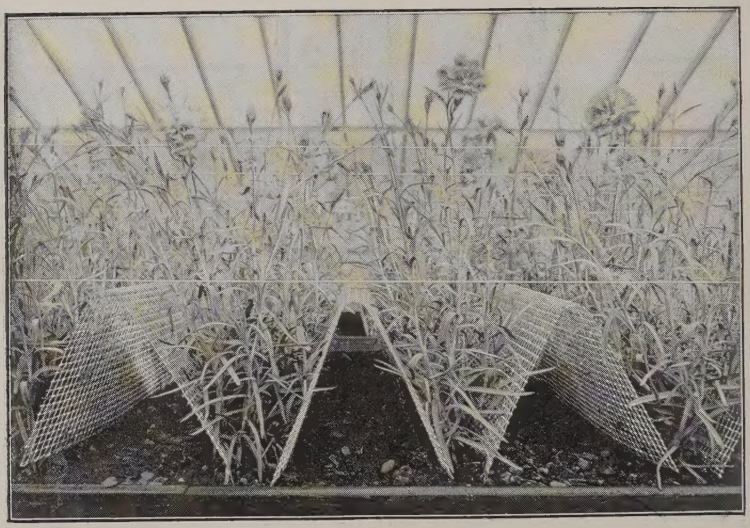


Fig. 24.

VIEWS IN MR. WARD'S CARNATION HOUSES.

Fig. 11. Typical form of *Bacterium Dianthi*. From agar-agar culture 40 days old. × about 2000.

Fig. 12. Growth forms in drop culture after 24 hours: *a* motile stage, *b* non-motile stage in which there is great activity of division, but the individuals are no longer free to move because of the secretion of a mucilaginous envelope, *c* temporary filaments, *d* zoogloea in the early stages of formation. × 700.

Plate IV.—Growth of *Bacterium Dianthi* in plate cultures. × 200.

Fig. 13. The form assumed on nutrient neutral gelatin after two days' growth. Colonies spreading, zoogloea in only moderate abundance, color a pale cream tint.

Fig. 14. The form assumed on strongly acid cultures, otherwise similar to the preceding. Two days' growth. Zoogloea far more abundant, color a light orange tint.

Plate V.—Formation of zoogloea in drop culture, × 700; three leaves of carnation, natural size.

Fig. 15. A portion of the field as seen in drop culture of corn (maize) infusion, after three days' growth. A very abundant formation of zoogloea.

Fig. 16. Healthy leaf of carnation.

Fig. 17. A carnation leaf, healthy except in the upper half where it shows bacteriosis from an application of a drop of fluid culture of the germ. Inoculated Dec. 10, 1889, drawn as it appeared six weeks later.

Fig. 18. A leaf badly affected with bacteriosis from a natural infection, showing later stages of the disease prior to withering of the leaf.

Plate VI—Cross sections of leaves magnified.

Fig. 19. Section through a carnation leaf after the cells have been partially disorganized by the prolonged action of bacteriosis, showing the inroads of *Bacillus subtilis* and other kinds of saprophytic bacteria, which complete the destruction begun by *Bacterium Dianthi*. The stomatal passages are filled, and the adjacent surface of the leaf is overrun. × 400 diameters.

Fig. 20. Cross section of the leaf of Japan pink affected with bacteriosis from an artificial infection with *Bacterium Dianthi*; *a* passage of stoma filled with bacteria, *b* air chamber beneath stoma, *c* epidermal cells showing eroded walls, with bacteria closely appressed to the eroded surfaces, *d* parenchyma cells in which the chlorophyll grains are being rapidly disorganized by the bacteria, *e* partition walls between cells perforated and broken down by the bacteria, *f* cells yet remaining comparatively free from attack.

Plate VII.—Views in the carnation houses of Mr. Fred. Dorner, of LaFayette, Ind.

Fig. 21. General view of carnation house with short span to the north and low benches. Photographed in November, just after putting the wire netting in place and stretching the first level of strings. On the right hand side the supports are in place to hold the second level of strings.

Fig. 22. Side view of bench, showing the manner of placing the wire netting and height of the first level of strings.

Plate VIII.—Views in the carnation houses of Mr. C. W. Ward, of Queens, N. Y.

Fig. 23. General view of carnation house properly wired and netted. In the middle bench the plants have not yet reached the upper wires of the supports, while in the bench at the left the buds are above the upper wires.

Fig. 24. Side view of bench showing exact method of bending and placing the netting, and its efficiency.